EpiNext™ High-Sensitive DNA Library Preparation Kit (Illumina)

Base Catalog # P-1053

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

Uses: The EpiNext™ High Sensitive DNA Library Preparation Kit (Illumina) is suitable for preparing a DNA library using sub-nanogram amounts of DNA input for next generation sequencing applications using an Illumina sequencer. These applications include genomic DNA-seq, ChIP-seq, MeDIP/hMeDIP-seq, classical bisulfite-seq, and targeted re-sequencing. The optimized protocol and components of the kit allow both non-barcoded (singleplexed) and barcoded (multiplexed) DNA libraries to be constructed quickly with reduced bias.

Starting Material and Input Amount: Starting materials can include fragmented dsDNA isolated from various tissue or cell samples, dsDNA enriched from a ChIP reaction, MeDIP/hMeDIP reaction or exon capture. DNA should be relatively free of RNA because large fractions of RNA will impair end repair and dA-tailing, resulting in reduced ligation capabilities. The input amount of DNA can be from 0.2 ng to 100 ng. For optimal preparation, the input amount should be 10 ng to 50 ng.

Precautions: To avoid cross-contamination, carefully pipette the sample or solution into the tube/vials. Use aerosol-barrier pipette tips and always change pipette tips between liquid transfers. Wear gloves throughout the entire procedure. In case of contact between gloves and sample, change gloves immediately.

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KIT CONTENTS

Component	12 reactions Cat. #P-1053-12	24 reactions Cat. #P-1053-24	Storage Upon Receipt
10X End Polishing Buffer*	30 µl	60 µl	-20°C
End Polishing Enzyme Mix*	13 µl	26 µl	-20°C
End Polishing Enhancer	13 µl	26 µl	-20°C
2X Ligation Buffer*	250 µl	500 µl	-20°C
T4 DNA Ligase*	15 µl	30 µl	-20°C
Adaptors (50 µM)*	15 µl	30 µl	-20°C
MQ Binding Beads*	1.6 ml	3.2 ml	4°C
2X HiFi PCR Master Mix*	160 µl	320 µl	-20°C
Primer U (10 µM)*	15 µl	30 µl	-20°C
Primer I (10 µM)*	15 µl	30 µl	-20°C
Elution Buffer*	1000 µl	2000 µl	-20°C
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** Spin the solution down to the bottom prior to use.*

SHIPPING & STORAGE

The kit is shipped on frozen ice packs at 4°C.

Upon receipt: Store the following components at -20°C immediately: **10X End Polishing Buffer**, **End Polishing Enzyme Mix**,**End Polishing Enhancer**, **2X Ligation Buffer**, **T4 DNA Ligase**, **Adaptors**, **2X HiFi PCR Master Mix**, **Primer U**, **Primer I**, and **Elution Buffer**. Store the following components at 4°C: **MQ Binding Beads.** Store all other components at room temperature.

MATERIALS REQUIRED BUT NOT SUPPLIED

- ☐ Vortex mixer
- ☐ Sonicator or enzymes for DNA fragmentation
- ☐ Agilent® Bioanalyzer® or comparable method to assess the quality of DNA library
- ☐ Thermocycler
- ☐ Centrifuge including desktop centrifuge (up to 14,000 rpm)
- ☐ Magnetic stand (96-well format)
- ☐ Pipettes and pipette tips
- ☐ PCR tubes or plates
- ☐ 1.5 ml microcentrifuge tubes
- ☐ 80% Ethanol

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- ☐ Distilled water
- ☐ DNA sample

GENERAL PRODUCT INFORMATION

Quality Control: Each lot of EpiNext™ High Sensitive DNA Library Preparation Kit (Illumina) is tested against predetermined specifications to ensure consistent product quality. Epigentek guarantees the performance of all products in the manner described in our product instructions.

Product Warranty: If this product does not meet your expectations, simply contact our technical support unit or your regional distributor. We also encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

Safety: Suitable lab coat, disposable gloves, and proper eye protection are required when working with this product.

Product Updates: Epigentek reserves the right to change or modify any product to enhance its performance and design. The information in this User Guide is subject to change at any time without notice. Thus, only use the User Guide that was supplied with the kit when using that kit.

Usage Limitation: The EpiNext™ High Sensitive DNA Library Preparation Kit (Illumina) is for research use only and is not intended for diagnostic or therapeutic application.

Intellectual Property: The EpiNext™ High Sensitive DNA Library Preparation Kit (Illumina) and methods of use contain proprietary technologies by Epigentek.

A BRIEF OVERVIEW

DNA library preparation is a critical step for next generation sequencing (NGS). For generating accurate sequencing data in NGS, the prepared library DNA should be sufficient in yield and of high quality. Also as NGS technology is continuously improving, DNA library preparation is required to be optimized accordingly. For example, most of the currently used methods are time-consuming, expensive, inconvenient, and specifically need large amounts of DNA. These reactions result in a DNA library preparation which cannot be used for biological samples with limited amounts of starting material such as tumor biopsy, early embryos, embryonic tissues and circulating DNA. In addition, the amount of DNA enriched by ChIP or MeDIP/hmeDIP is often at low or sub-nanogram levels which causes insufficient DNA library yields. To address this issue, Epigentek offers the EpiNext™ High Sensitive DNA Library Preparation Kit (Illumina). This kit has the following features:

- High sensitivity and flexibility: Can be used for both non-barcoded (singleplexed) and barcoded (multiplexed) DNA library preparation. The amount of input DNA can be as low as 0.2 ng with a range from 0.2 to 100 ng. Various dsDNA can be used, which includes limited amounts of fragmented dsDNA isolated from various tissue or cell samples, dsDNA enriched from ChIP reactions, and dsDNA enriched from MeDIP/hMeDIP reactions or exon capture.
- Fast and streamlined procedure: the procedure from fragmented DNA to size selection is less than 1 h 30 min. No clean-up is required between each step and all reactions take place in the same tube, thereby saving time and preventing handling errors, as well as loss of valuable samples. Gel-free size selection further reduces the preparation time.

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- The most convenient for use: the kit contains all required components for each step of DNA library preparation, which are sufficient for end polishing, ligation, clean-up, size selection and library amplification, thereby allowing the library preparation to be the most convenient with reliable and consistent results.
- Minimized bias: Ultra HiFi amplification enables to reproducibly achieve high yields of DNA library with minimal sequence bias and low error rates.

PRINCIPLE & PROCEDURE

The EpiNext™ High Sensitive DNA Library Preparation Kit (Illumina) contains all reagents required at each step of workflow for carrying out successful DNA library preparation. In the library preparation, DNA is first fragmented to appropriate size (about 300 bps in peak size). The end repair/dA tailing (end polishing) of the DNA fragments are performed simultaneously. Adaptors are then ligated to both ends of the polished DNA fragments for amplification and sequencing. Ligated fragments are size selected and purified with MQ beads, which allows quick and precise size selection of DNA. Size-selected DNA fragments are amplified with high-fidelity PCR Mix that ensures maximum yields from minimum amounts of starting material and provides highly accurate amplification of library DNA with low error rates and minimum bias.

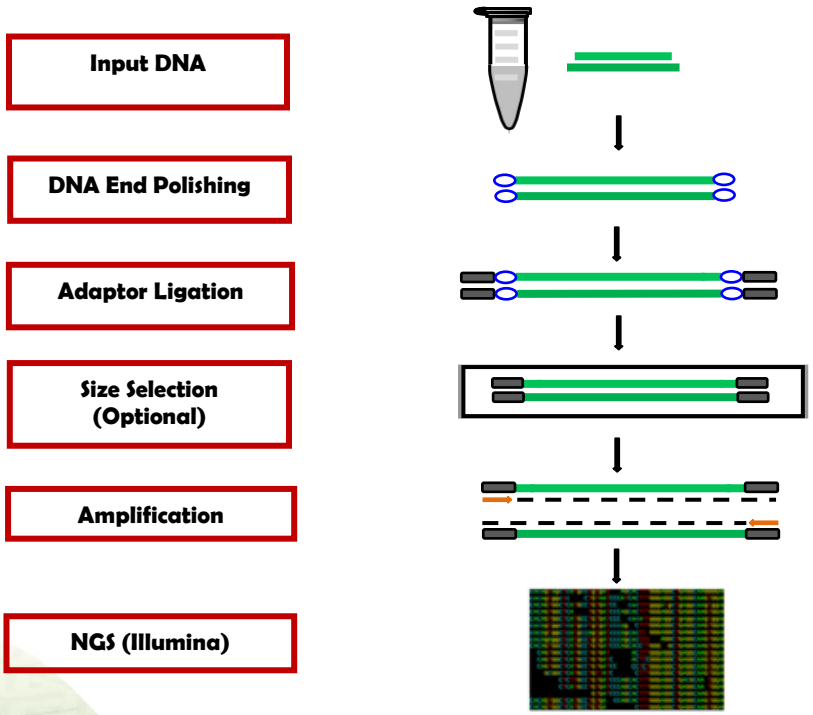

Fig. 1. Workflow of the EpiNext™ High Sensitive DNA Library Preparation Kit

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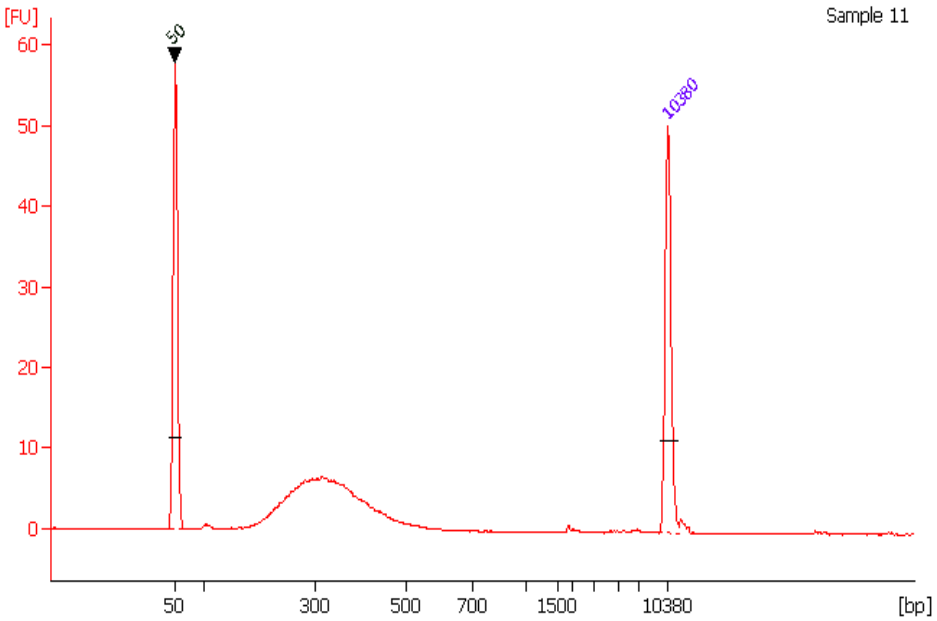

Fig2. Size distribution of library fragments. Human placenta DNA was sheared to around 300 bps in peak size and 0.2 ng of DNA was used for DNA library preparation using EpiNext™ High Sensitive DNA Library Preparation Kit (Illumina)

ASSAY PROTOCOL

For the best results, please read the protocol in its entirety prior to starting your experiment.

Starting Materials

Fragmented dsDNA that is isolated from various tissues or cell samples: 0.2 ng-100 ng, optimized 10-50 ng per preparation.

dsDNA enriched from a ChIP reaction, MeDIP/hMeDIP reaction or exon capture: 0.2 ng-100 ng. DNA should be of high quality and relatively free of RNA. RNAse I can be used to remove RNA and DNA should be eluted in DNase/RNase-free water.

DNA Fragmentation

dsDNA enriched from a ChIP reaction, MeDIP/hMeDIP reaction or exon capture should already be fragmented. DNA isolated from various tissue or cell samples can be fragmented using one of the following methods. For the best results we highly recommend using a waterbath-based sonication

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device. The peak size of fragmented DNA should be compatible with the read length of the Illumina sequencing platform to be used. In general the peak size of fragments should be 200-300 bps.

Waterbath Sonication:

Epigentek's EpiSonic 1100 (Epigentek Cat No. EQC-1100): For target peak size 200 bps, use 10 µl of DNA solution (standard amount 10-50 ng) per 0.2 ml PCR tube. Shear 40 cycles under cooling condition, 45 seconds On, 15 seconds Off, each at 110-120 watts. For more detailed information of use, please see the "DNA Shearing Protocol" for EpiSonic 1100.

If using other waterbath sonicators please follow the supplier's instruction.

Enzymatic Shearing:

The DNA can also be sheared using various enzyme-based methods. Optimization of the shearing conditions, for example enzyme concentration and incubation time, is needed in order to use enzyme-based methods.

1. DNA End Polishing

a. Prepare end repair reaction in a 0.2 ml PCR tube according to Table 1:

Table 1. End Polishing Reaction

Component	Volume
Fragmented DNA	10 µl (10-50 ng)
10X End Polishing Buffer	1.5 µl
End Polishing Enzyme Mix	1 µl
End Polishing Enhancer	1 µl
Distilled Water	1.5 µl
Total Volume	**15 µl**

b. Mix and incubate for 20 min at 25°C and 20 min at 72°C in a thermocycler (without heated lid).

***Note**: the amount of fragmented DNA can be 0.2-100 ng with an optimal amount of 10-50 ng.*

2. Adaptor Ligation

a. Prepare a reaction mix for adaptor ligation according to Table 1. Add the following reagents to a 0.2 ml PCR tube containing end polished DNA from step 1.

Table 2. Adaptor Ligation

Component	Volume
End polished DNA (from step 1)	15 µl
2X Ligation Buffer	17 µl
T4 DNA Ligase	1 µl
Adaptors	1 µl
Total Volume	**34 µl**

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b. Mix and incubate for 15 min at 25°C in a thermocycler (without heated lid).

Note: *(1) The pre-annealed adapters included in the kit are suitable for both non-barcoded (singleplexed) and barcoded (multiplexed) DNA library preparation and are fully compatible with Illumina platforms, such as MiSeq® or HiSeq™ sequencers. (2) If using adaptors from other suppliers (both single-end and barcode adaptors), make sure they are compatible with Illumina platforms and add the correct amount (final concentration 1.5-2 µM, or according to the supplier's instruction).*

3. Size Selection/Clean-up

3.1. Size selection of Ligated DNA

Note*: If the starting DNA amount is less than 50 ng, size selection is not recommended and alternatively, clean-up of ligated DNA can be performed prior to PCR amplification according to step 3.2 of the protocol.*

a. Resuspend **MQ Binding Beads** by vortex.
b. Add 14 µl of resuspended **MQ Binding Beads** to the tube of ligation reaction. Mix well by pipetting up and down at least 10 times.
c. Incubate for 5 minutes at room temperature.
d. Put the tube on an appropriate magnetic stand until the solution is clear (about 4 minutes). Carefully transfer the supernatant containing DNA to a new tube (**Caution: do not discard the supernatant**). Discard the beads that contain the unwanted large fragments.
e. Add 10 µl resuspended beads to the supernatant, mix well and incubate for 5 minutes at room temperature.
f. Put the PCR tube on an appropriate magnetic stand until the solution is clear (about 4 minutes). Carefully remove and discard the supernatant. **Be careful not to disturb or discard the beads that contain DNA.**
g. Keep the PCR tube in the magnetic stand and add 200 µl of freshly prepared 80% ethanol to the tube. Incubate at room temperature for 1 min, and then carefully remove and discard the ethanol.
h. Repeat Step 3.1g one time, for total of two washes.
i. Open the PCR tube cap and air dry beads for 10 minutes while the tube is on the magnetic stand.
j. Resuspend the beads in 12 µl **Elution Buffer**, and incubate at room temperature for 2 minutes to release the DNA from the beads.
k. Capture the beads by placing the tube in the magnetic stand for 4 minutes or until the solution is completely clear.
l. Transfer 11 µl to a new 0.2 ml PCR tube for PCR amplification.

3.2. Clean-up of Ligated DNA (Optional)

a. Resuspend **MQ Binding Beads** by vortex.
b. Add 34 µl of resuspended beads to the PCR tube of ligation reaction. Mix thoroughly on a vortex mixer or by pipetting up and down at least 10 times.
c. Incubate for 5 minutes at room temperature to allow DNA to bind to beads.
d. Put the PCR tube on an appropriate magnetic stand until the solution is clear (about 4 minutes). Carefully remove and discard the supernatant. **Be careful not to disturb or discard the beads that contain DNA**.
e. Keep the PCR tube in the magnetic stand and add 200 µl of freshly prepared 80% ethanol to the tube. Incubate at room temperature for 1 min, and then carefully remove and discard the ethanol.

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f. Repeat Step 3.2e two times for total of three washes.
g. Open the PCR tube cap and air dry beads for 10 minutes while the tube is on the magnetic stand.
h. Resuspend the beads in 12 µl **Elution Buffer**, and incubate at room temperature for 2 minutes to release the DNA from the beads.
i. Capture the beads by placing the tube in the magnetic stand for 4 minutes or until the solution is completely clear.
j. Transfer 11 µl to a new 0.2 ml PCR tube for PCR amplification.

4. Library Amplification

a. *Prepare the PCR Reactions*

Thaw all reaction components including master mix, DNA/RNA free water, primer solution and DNA template. Mix well by vortexing briefly. Keep components on ice while in use, and return to –20˚C immediately following use. Add components into each PCR tube/well according to the following table:

Component	Size (µl)
HiFi Master Mix (2X)	12.5 µl
Primer U	1 µl
Primer I (or barcode)	1 µl
Adaptor Ligated DNA	10.5 µl
Total Volume	**25 µl**

Important Note: *Use of **Primer I** included in the kit will generate a singleplexed library. For multiplexed library preparation, replace **Primer I** with one of the12 different barcodes (indexes) contained in the EpiNext™ NGS Barcode (Index) Set-12 (Cat. No. P-1060). You can also add user-defined barcodes (Illumina compatible) instead of **Primer I**.*

b. *Program the PCR Reactions*

Place the reaction plate in the instrument and set the PCR conditions as follow:

Cycle Step	Temp	Time	Cycle
Activation	98°C	30 sec	1
Cycling	98°C 55°C 72°C	20 sec 20 sec 20 sec	Variable*
Final Extension	72°C	2 min	1

** PCR cycles may vary depending on the input DNA amount. In general, use 10 PCR cycles for 100 ng, 12 cycles for 50 ng, 16 cycles for 5 ng, 18 cycles for 1 ng and 22 cycles for 0.2 ng DNA input. Further optimization of PCR cycle number may be required.*

5. Clean-up of Amplified Library DNA

a. Resuspend **MQ Binding Beads** by vortex.

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b. Add 25 µl of resuspended beads to the PCR tube of amplification reaction. Mix thoroughly on a vortex mixer or by pipetting up and down at least 10 times.
c. Incubate for 5 minutes at room temperature to allow DNA to bind to beads.
d. Put the PCR tube on an appropriate magnetic stand until the solution is clear (about 4 minutes). Carefully remove and discard the supernatant. **Be careful not to disturb or discard the beads that contain DNA**.
e. Keep the PCR tube in the magnetic stand and add 200 µl of freshly prepared 80% ethanol to the tube. Incubate at room temperature for 1 min, and then carefully remove and discard the ethanol.
f. Repeat Step 7e two times for a total of three washes.
g. Open the PCR tube cap and air dry beads for 10 minutes while the tube is on the magnetic stand.
h. Resuspend the beads in 22 µl **Elution Buffer**, and incubate at room temperature for 2 minutes to release the DNA from the beads.
i. Capture the beads by placing the tube in the magnetic stand for 4 minutes or until the solution is completely clear.
j. Transfer 20 µl to a new 0.2 ml PCR tube.

Quality of the prepared library can be assessed using an Agilent Bioanalyzer or comparable method. Library fragments should have the correct size distribution (ex: 300 bps at peak size) without adaptors or adaptor-dimers. To check the size distribution, dilute library 5-fold with water and apply it to an Agilent high sensitivity chip. If there is presence of <150 bp adaptor dimers or of larger fragments than expected, they should be removed. To remove fragments below 150 bps use 0.8X **MQ Binding Beads** (ex: add 16 µl of **MQ Binding Beads** to 20 µl of sample) according to steps a-i of section **5. Clean-up of Amplified Library DNA.** To remove fragments above 500 bps follow steps a-l of section **3.2 Size Selection of Ligated DNA**.

The prepared DNA library can be quantified with various DNA library quantification methods.

The prepared library DNA can be stored at -20˚C until ready to use for sequencing.

TROUBLESHOOTING

Problem	Possible Cause	Suggestion
Low yield of library	Insufficient amount of starting DNA.	To obtain the best results, the amount of input DNA should be >10 ng.
	Insufficient purity of starting DNA.	Ensure that RNA is removed by Rnase treatment before starting library preparation protocol.
	Improper reaction conditions at each reaction step.	Check if the reagents are properly added and incubation temperature and time are correct at each reaction step including End Polishing, Adaptor Ligation, size Selection and Amplification.
	Improper storage of the kit	Ensure that the kit has not exceeded the expiration date. Standard shelf life, when stored properly, is 6 months from date of receipt.
Unexpected peak size	Improper ratio of **MQ Binding**	Check if the correct volume **of MQ**

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of Agilent bioanalyzer trace: Presence of <150 bp adaptor dimers or presence of larger fragments than expected.	**Beads** to DNA volume in size selection	**Binding Beads** is added to the DNA solution accordingly. Proper ratios should remove the fragments of unexpected peak size
	Insufficient ligation	Too much and too little input DNA may cause insufficient ligation, which can shift the peak size of the fragment population to be shorter or larger than expected. Make sure that the ligation reaction is properly processed with proper amount of input DNA.
	Over-amplification of library	PCR artifacts from over-amplification of the library may cause the fragment population to shift higher than expected. Make sure to use proper PCR cycles to avoid this problem.

RELATED PRODUCTS

DNA Isolation and Cleanup

P-1003	FitAmp™ General Tissue Section DNA Isolation Kit
P-1004	FitAmp™ Plasma/Serum DNA Isolation Kit
P-1006	DNA Concentrator Kit
P-1007	FitAmp™ Gel DNA Isolation Kit
P-1009	FitAmp™ Paraffin Tissue Section DNA Isolation Kit
P-1017	FitAmp™ Urine DNA Isolation Kit
P-1018	FitAmp™ Blood and Cultured Cell DNA Extraction Kit

Sonication Instruments

EQC-1100	EpiSonic™ Multi-Functional Bioprocessor 1100

DNA Enrichment Reaction

P-1015	Methylamp™ Methylated DNA Capture Kit
P-1038	EpiQuik™ Hydroxymethylated DNA Immunoprecipitation (hMeDIP) Kit
P-1052	EpiQuik™ MeDIP Ultra Kit
P-2002	EpiQuik™ Chromatin Immunoprecipitation Kit
P-2003	EpiQuik™ Tissue Chromatin Immunoprecipitation Tissue Kit
P-2014	EpiQuik™ Plant ChIP Kit
P-2025	ChromaFlash™ One-Step ChIP Kit
P-2026	ChromaFlash™ One-Step Magnetic ChIP kit
P-2027	ChromaFlash™ ChIP Ultra Kit

DNA Bisulfite Conversion

P-1001	Methylamp™ DNA Modification KIt
P-1026	BisulFlash™ DNA Modification Kit

PCR Analysis

P-1028	Methylamp™ MS-qPCR Fast Kit
P-1029	EpiQuik™ Quantitative PCR Fast Kit

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DNA Library Prep

P-1051	EpiNext™ DNA Library Preparation Kit
P-1055	EpiNext™ Post-Bisulfite DNA Library Preparation Kit (Illumina)

NGS Barcode

P-1060	EpiNext™ NGS Barcode (Index) Set-12

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